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TITLE: Assay preparation containing capture and detection polynucleotides covalently bound to substrates with a heterobifunctional crosslinking agent

Brief Summary Text (11):
It is an essential feature of nucleic acid absorption to substrates that the strands must be denatured to efficiently immobilize. Accordingly, mobilization often interferes with the ability of a nucleic acid sequence to hybridize in an assay. DNA is commonly immobilized by absorption to nitrocellulose, nylon, or hyrdoxyapatite. In order for the DNA to remain insolubilized throughout the assay the strand must be of sufficient length to form several attachment points, this form of absorption often slows the ability of immobilized target DNA to efficiently reanneal with labeled probe DNA. Low molecular weight oligonucleotides and RNA are usually immobilized through a process of absorption and covalent attachment to nylon membranes. For covalent attachment to occur amines on the target sequence must be crosslinked to amines on the support. For single strand DNA, RNA and oligonucleotides the free amines of adenine, guanine, and cytosine are often utilized, rendering these bases unavailable for use in forming interstrand diagnostic interactions with labeled probe. Several previous attempts have utilized artificial nucleotides that have free amine groups available for crosslinking. These molecules must be added to the probe strand prior to linking to the amine on the support. One example employs synthetic nucleotide triphosphates with aminoalkyl function groups extending from the base, added to the 5' end of a DNA chain using polynucleotide kinase. This form of an attachment has some use for double strand DNA where amine bearing residues are blocked by interstrand hydrogen bonds, but offers little advantage for single strand DNA and RNA. Covalent crosslinking of polynucleotides to nylon membranes is also accomplished by generation of photoadducts. This process involves the separate steps of absorbing DNA or RNA to a nylon membrane, drying the membrane and using ultraviolet light (usually 254 nm wavelength) to nonspecifically attach bases to the free amines of the nylon. Covalent attachment of nucleotides often renders the DNA useless for diagnostic purposes. For example, crosslinking of amines and carboxylic acids using carbodiimide methods that work well for proteins will render nucleic acids insoluble through interstrand covalent bonds. Aldehydes, such as glutaraldehdye, can attach to hydroxyl groups throughout the length of the polymer's sugar backbone rather than at specific loci, and may crosslink strands together preventing annealing with a labeled probe sequence.